Dietary supplement attenuates radiation-induced osteoclastogenic and oxidative stress-related responses and protects adult mice from radiation-induced bone loss

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Abstract

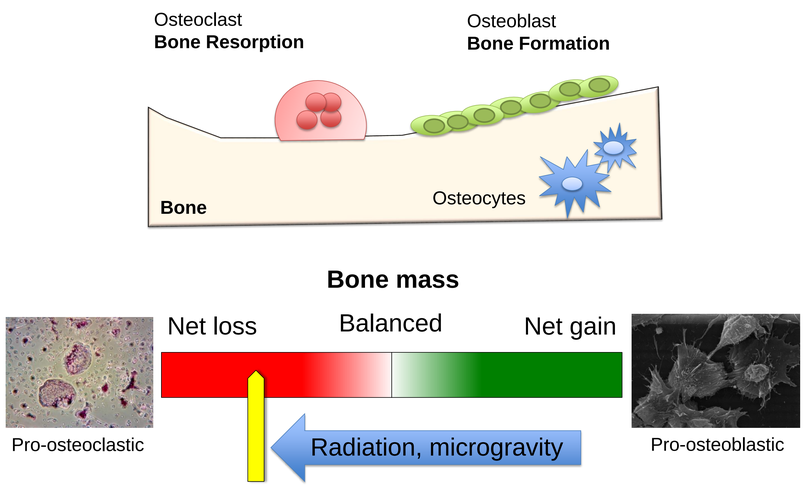
Our **central hypothesis** is that oxidative stress plays a key role in cell dysfunction and progressive bone loss caused by radiation exposure during spaceflight. In animal studies, excess free radical formation is associated with pathological changes in bone structure, enhanced bone resorption, reduced bone formation and decreased bone mineral density, which can lead to skeletal fragility. We previously reported that exposure to low or high-LET radiation rapidly increases expression levels of pro-osteoclastogenic and oxidative stress-related genes in bone and marrow, followed by pathological changes in skeletal structure. To screen various antioxidants for radioprotective effects on bone, 4 month old, male C57Bl6/J mice were treated with a dietary antioxidant cocktail, injectable α-lipoic acid, or a dried plum-enriched diet (DP). Mice were then exposed to 2Gy ^{137}Cs total body radiation and one day later marrow cells were collected and the relevant genes analyzed for expression levels. Of the candidates tested, DP was most effective in reducing bone resorption-related gene expression. Microcomputed tomography revealed that DP also prevented the radiation-induced deterioration of skeletal microarchitecture, as indicated by percent bone volume, trabecular spacing and trabecular number. DP had similar protective effects on skeletal structure after sequential exposure to protons (0.5 Gy, 150MeV/n) and ^{56}Fe 0.5Gy, 600 MeV/n). When cultured ex vivo under osteogenic conditions, bone marrow-derived cells from DP-fed animals exhibited increased colony numbers compared to control diet-fed animals. These findings suggest that DP exerted pro-osteogenic effects apart from previously identified anti-resorptive actions, which may contribute to radioprotection of skeletal tissue. In conclusion, a diet enriched in certain types of antioxidants and polyphenols such as DP may be useful as an intervention to protect tissues from degenerative effects of ionizing radiation.

Purpose of the study

- Assess the ability of selected antioxidants to mitigate radiation-induced bone loss
- Determine the mechanisms underlying radiation-induced bone loss

Overview

High doses of radiation lead to progressive bone loss



Experiment design

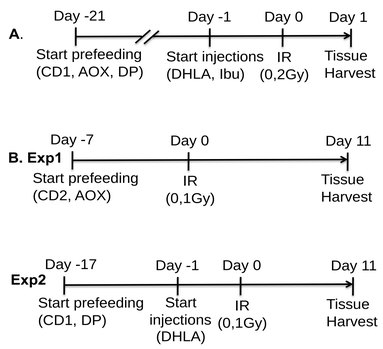


Figure 1. Male mice were exposed to total body irradiation (TBI) at 16 weeks of age. Panel A shows the short-term gene expression experiment. Mice were pre-fed for 21 days with the diets (CD1, AOX or DP), or injected twice a day with DHLA or Ibuprofen starting one day prior to TBI. In Panel B, mice were pre-fed for 14 days with control diet (CD3) or DP diet and then irradiated at 16 weeks with 2 Gy Gamma. In Panel C Exp. 1, mice were pre-fed with CD1 or DP at 17 days before irradiation. DHLA was injected at 12-hour intervals one day before TBI until tissue harvest. In Exp. 2, mice were fed AOX or control diet 2 (CD2) beginning at 7 days before TBI. Tissues were harvested at time points indicated above.

Results

Assessment of total antioxidant capacity

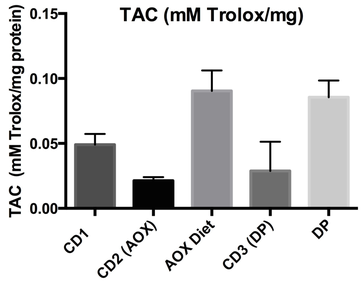


Figure 2. Dietary total antioxidant capacity (TAC) was measured in the diets used in the study. CD1 (Purina 5001) is the standard diet, CD2 (AIN93G), is the control for the AOX-supplemented diet and CD3 (AIN-93M) is the control for DP. Data shown are mean ±S.D. from 3-4 separate aliquots.

DP diet mitigates radiation-induced up-regulation of pro-osteoclastogenic and oxidative response markers in bone marrow

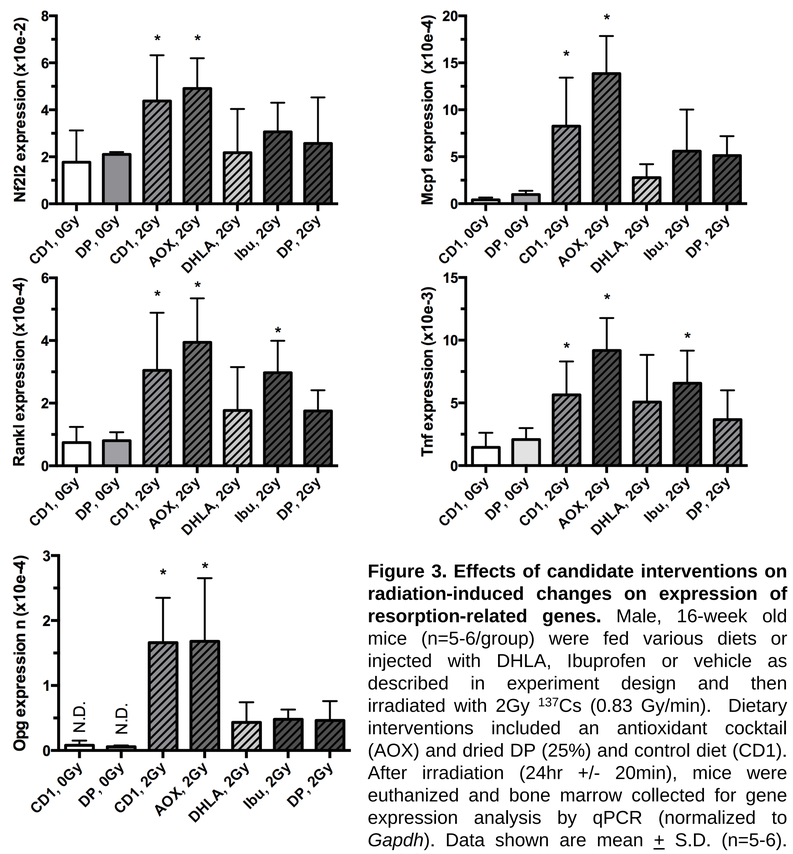


Figure 3. Effects of candidate interventions on radiation-induced changes on expression of resorption-related genes. Male, 16-week old mice (n=5-6/group) were fed various diets or injected with DHLA, Ibuprofen or vehicle as described in experiment design and then irradiated with 2Gy ^{137}Cs (0.83 Gy/min). Dietary interventions included an antioxidant cocktail (AOX) and dried DP (25%) and control diet (CD1). After irradiation (24hr +/- 20min), mice were euthanized and bone marrow collected for gene expression analysis by qPCR (normalized to *Gapdh*). Data shown are mean ± S.D. (n=5-6). *p<0.05 compared to mice fed on control diet, sham irradiated.

DP diet rescues radiation-induced decrements in skeletal microarchitecture

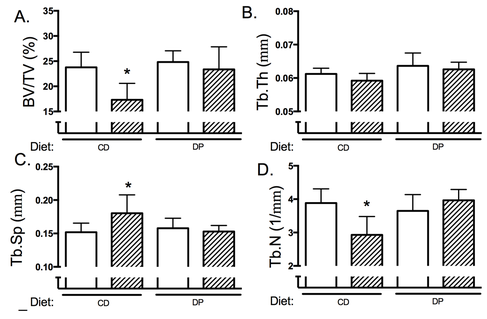


Figure 4. Effects of DP on gamma radiation-induced cancellous bone loss
Sixteen-week old male mice were pre-fed with control (CD3) or DP diet, exposed to 2Gy ^{137}Cs and euthanized at 11 days post-IR (see Experiment Design). Bones were analyzed by microcomputed tomography (microCT) for percent bone volume (BV/TV, Panel A), trabecular thickness (Tb.Th, Panel B), trabecular separation (Tb.Sp, Panel C) and trabecular number (Tb.N, Panel D). Data shown are mean ± S.D. (n=8/group). *P<0.05 compared to control diet/sham irradiated group.

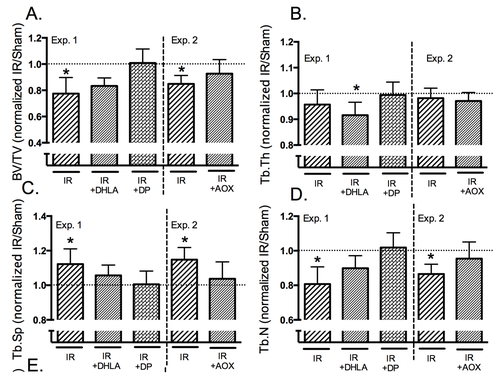


Figure 5. Effects of selected interventions on bone loss induced by simulated spaceflight
Sixteen-week old male mice were pre-fed with selected diets or injected with DHLA as followed by irradiation with 1 Gy dual ion (Protons and Iron) in two separate experiments labeled Expt #1 and Exp #2 (see Experiment)Design. Mice were euthanized 11 days post-IR and bones analyzed by microCT as described in Figure 4. Data is shown normalized to its own sham (non-irradiated) control group. Data shown are mean ± S.D. (n=8-10). *P<0.05 compared to corresponding control diet/sham irradiated control.

DP diet preserves ex vivo mineralization ability of radiation-challenged osteoblasts

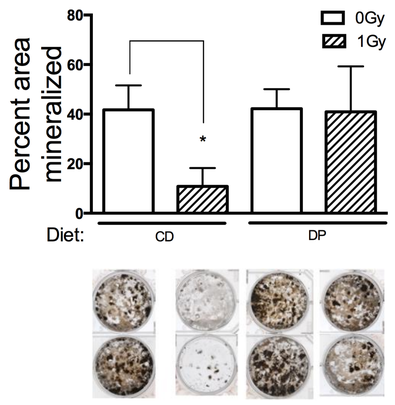


Figure 6. Bone marrow-derived osteoblast cultures retain mineralization capacity radiation.
Sixteen-week old male mice pre-fed with control (CD) or DP diet for 17 days and euthanized. Marrow from femora and tibiae were flushed and cultured in osteogenic medium. At day 3 in culture, cells were irradiated with 1 Gy ^{56}Fe. At day 21 in culture, cells were fixed followed by Von Kossa staining (lower panel). Quantification of extent of mineralization was performed (upper panel, n=XX/group). *P<0.05 compared to non-irradiated control.

Conclusions

- Radiation induces acute and persistent damage to bone and associated tissue.
- Radiation-induced bone loss is to some extent driven by the early increase in bone resorption response as well as oxidative stress and that and the capacity to prevent these early responses can effectively mitigate bone loss.
- These early markers are useful tools to assess and screen for candidate interventions against bone loss.
- Plum supplementation can positively alter the skeletal response to radiation

Acknowledgement

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